



## ProFoldin

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## INSTRUCTIONS

# ProFoldin

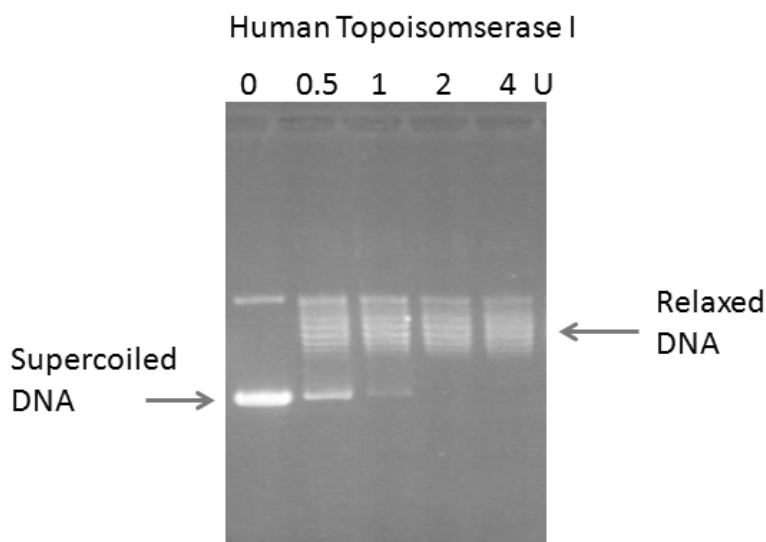
# Gel-Based Human Topoisomerase I DNA relaxation Assay Kit

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Catalog No. GHRA100KE

## Introduction

Human DNA topoisomerase I (topo I) converts supercoiled circular DNA into relaxed DNA (DNA relaxation reaction). The **Gel-Based Human Topoisomerase I DNA relaxation Assay Kit** is based on the principle that the supercoiled DNA and relaxed DNA are separated by agarose gel electrophoresis. Fluorescence-based DNA relaxation assays in a 96-well plate format are also available for high throughput screening of human topo I inhibitors. For more information of the high throughput human topo I assays, please visit the website at <http://www.profoldin.com>.



The **Gel-Based Human Topoisomerase I DNA relaxation Assay Kit** (Catalog No. GHRA100KE) includes all the reagents for 100 samples in gel-based assays of human topo I DNA relaxation activity. It includes 400  $\mu$ l of 10 x Buffer HT1, 100  $\mu$ l of 250  $\mu$ g/ml supercoiled DNA, 550  $\mu$ l of 5 x Gel loading buffer and 10  $\mu$ l of 500 U/ $\mu$ l human topoisomerase I enzyme.

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# INSTRUCTIONS

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## Assay Protocol

### 1. Reaction:

The total volume of each reaction mixture is 20  $\mu$ l in an eppendorf tube including: 16  $\mu$ l of H<sub>2</sub>O, 2  $\mu$ l of 10 x Buffer HT1, 1  $\mu$ l of 250  $\mu$ g/ml supercoiled DNA, 1  $\mu$ l of 50 U/ $\mu$ l human topo I. Incubate the reaction mixture at 37°C for 60 min. At the end of the reaction, add 5  $\mu$ l of 5 x gel loading buffer.

Note: The final concentrations are 25 mM Tris-Cl, pH 7.4, 57 mM KCl, 5 mM MgCl<sub>2</sub>, 0.25 mM DTT, 0.25 mM EDTA and 15  $\mu$ g/ml BSA, 12.5  $\mu$ g/ml supercoiled plasmid DNA and 2.5 U/ $\mu$ l human topo I. The 50 U/ $\mu$ l enzyme solution is prepared freshly by dilution of the 500 U/ $\mu$ l stock solution in 1x assay buffer. The diluted enzyme may not be stable for storage. For inhibitor IC<sub>50</sub> measurements, assay linearity should be tested to determine the optimal enzyme amount for the assay.

### 2. Agarose gel electrophoresis

- (1) Prepare 1 % agarose gel in 1 x TAE buffer.
- (2) Load 25 ul of the sample.
- (3) Run the gel at 100 V for 90 min to 2 hours.
- (4) Stain the gel in an ethidium bromide solution and destain the gel in water.
- (5) Take a picture of the gel under UV light.

## Related Products:

Human Topoisomerase I, 10,000 Units	Catalog No. HTOPI-010
Human Topoisomerase I, 40,000 Units	Catalog No. HTOPI-040
Human Topoisomerase I, 100,000 Units	Catalog No. HTOPI-100
Supercoiled Plasmid DNA -1 mg	Catalog No. SDNA-1MG
Relaxed Plasmid DNA -1 mg	Catalog No. RDNA-1MG
H19 Dye for DNA Relaxation and Supercoiling Assays	Catalog No. DSA1000D
Human DNA Topoisomerase I Assay Kit	Catalog No. HRA020K
Human DNA Topoisomerase I Assay Kit-100	Catalog No. HRA100K
Human DNA Topoisomerase I Assay Kit Plus-100	Catalog No. HRA100KE
Human DNA Topoisomerase I Assay Kit Plus-1000	Catalog No. HRA1000KE
<i>E. coli</i> DNA Topoisomerase I Assay Kit Plus-100	Catalog No. DRA100KE
Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	Catalog No. HDC100KE
96-Well Human Topo II DNA Decatenation Assay Kit Plus	Catalog No. HDD96KE

For more information of high throughput DNA topoisomerase assays, please see website of The Topo World: [http://www.profoldin.com/topoisomerase\\_assays\\_1.html](http://www.profoldin.com/topoisomerase_assays_1.html). For more information of drug targets and enzyme assays, please visit [www.profoldin.com](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).